THE PROBABLE IDENTITY OF THE OPSONINS WITH THE NORMAL AGGLUTININS.

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In the body fluids of animals there exist certain substances which while exerting no bacteriolytic action, prepare bacteria for inception by the mobile phagocytes. These were discovered by Denys and Leclef,* but were apparently rediscovered by Wright and Douglas,† who named them opsonins. It is only after bacteria have been subjected to the action of the opsonins that they are ingested by the polymorphonuclear white blood corpuscles. The average number of bacteria englobed by a polynuclear corpuscle can be used to indicate the relative amount of opsonins in a serum. The opsonins appear to play an active part in immunity. Their activity was specially noted by Wright and Douglas in the susceptibility or non-susceptibility of individuals towards invasion by the pyogenic staphylococci. The blood serum of patients who were subject to boils, etc., was always found to be low in opsonic power towards staphylococci compared with the serum of normal individuals. By the regulated inoculation of staphylococcus vaccine, a patient could be protected against accidental invasion, and at the same time the opsonic power of his serum rose above the normal.

Blood serum can opsonise‡ a variety of bacteria. All the bacteria tested by Wright and Douglas were ingested by the mobile phagocytes after treatment with blood serum. These

* Cent. f. Bakt. xxiv. 685 (also La Cellule, 1895).

† Proc. Roy. Soc. lxxii. (No.483), 1903, p.357; and lxxiii. (490), 1904, 128.

‡ A non-specificity does not appear to be claimed by Wright and Douglas for the opsonins, as they always speak of "the opsonins." Bulloch and Atkin, on the other hand, refer to "the opsonin," from which it would appear that they consider there is only one, and that non-specific.

included staphylococci, pneumococci and the micro-organisms of plague, Malta fever, dysentery, diphtheria, xerosis, anthrax and cholera. *Bac. tuberculosis** is also capable of being opsonised.

The opsonins are thermolabile, being destroyed by an exposure to a temperature of 60° for 15 minutes. This was confirmed by Bulloch and Atkin,† who also showed that a longer exposure at a lower temperature had the same destructive effect. In their conclusions they write, "The action of heat is to destroy the opsonin, and not merely to convert it into a non-opsonisable modification."

Wright and Douglas, and also Bulloch and Atkin, worked upon the staphylococcus, and when the temperature of destruction of the opsonins is given at 60° it can only refer to staphylococcus opsonin. In their second paper, published before that of Bulloch and Atkin, Wright and Douglas show that the opsonins are not entirely destroyed by heat when bacteria other than the staphylococcus are examined. In fact, staphylococcus opsonin appears to be peculiar in being completely destroyed at so low a temperature as 60° in 15 minutes. Still this is given by these authors as the destructive temperature for the opsonins, and we must use it as a working basis.

The action of the opsonins is to prepare the bacteria for inception by the phagocytes. This might be accomplished in three ways. First, the capsule may be altered to a chemotactic modification; secondly, it may be dissolved; and thirdly, it may be covered by a film of a positively chemotactic precipitate.

As the bacteria appear quite normal after opsonisation, the capsule is probably not dissolved. It may be altered, or it may be covered. Neither alteration nor covering is visible, but the same can be said about the films upon bacteria that have been agglutinated.

I have already shown‡ that bacteria such as *Bac. typhi*, which have been agglutinated, are capable of being englobed by the

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* Proc. Roy. Soc. lxxiv. (499), 159; and Urwick, Brit. Med. Journ., July 22nd, 1905, p.172.

† Proc. Roy. Soc. lxxiv. (504), 1905, 379.

‡ *Antea* p. 289.

polynuclear leucocytes, towards which in the normal or untreated state they are indifferent. In this respect, therefore, the opsonins are identical with the agglutinins. They both prepare the bacteria for inception by the phagocytes.

The similar behaviour of opsonised and agglutinated bacteria leads one to believe that after all there may be a close analogy between opsonin and agglutinin, and that certain of the points of difference might disappear upon further examination.

We know that blood serum normally contains small quantities of various agglutinins, but to make the matter certain, especially with regard to the staphylococcus agglutinins, I examined normal serum, my own, and found a decided agglutination for *Micr. aureus* with serum diluted from 3 to 200 times. Suspensions of dead staphylococci which had been killed by heat were also agglutinated.*

The chief difference between the two is found in the action of heat. The opsonins are destroyed at 60°, but there is some diversity of opinion regarding the effect of an exposure at that temperature upon the agglutinins. This is without doubt due to the fact that not only are the agglutinins specific with regard to their affinities for the products of particular bacteria, but they also differ in their behaviour to physical agents such as heat. According to Scheller, normal equine serum contains marked quantities of typhoid agglutinin which is scarcely affected by an exposure to a temperature of 62° for 30 minutes. Human typhoid agglutinin is considerably altered upon heating it at 60° for 15 minutes. In two cases which I tested, the agglutinating powers were reduced to one-seventh and one-tenth respectively. With regard to the normal agglutinins, which occur ordinarily

* It may be mentioned in this relation that while Wright and Douglas found that heating the staphylococcus suspension to 115° had no influence upon the behaviour of the bacteria towards the opsonins, Bulloch and Atkin demonstrated a reduction of the opsonic action after the bacteria had been heated for longer periods at high temperatures; for example an exposure for 60 minutes at 100° reduced the phagocytic index (the average number of cells englobed by a polynuclear leucocyte under the conditions of experiment) from 30 to 20.

in the human blood, we know little or nothing beyond the fact that they are present in relatively small amount.

It is generally supposed that the effect of moderate heat upon the agglutinins is to partially destroy them, but Dreyer found that *coli* agglutinin which had lost much of its power by heating exhibited its full power if the agglutination test was prolonged for 24 hours, and from this he concluded that the action of heat consisted in a slowing of the reaction between the agglutinin and the bacterial agglutinable substance. I believe this to be the true explanation. Typhoid agglutinin is certainly not destroyed, for I found that suspensions of typhoid bacteria treated with heated agglutinating serum beyond the limiting ratio for the heated serum were sedimented easily by the centrifuge, while neither normal suspensions nor those treated with unheated serum beyond the limiting ratio were so easily precipitated.

Those who have worked with opsonins have not examined the time factor in opsonisation, and it occurred to me that if instead of 15 minutes, 24 hours were given for the heated opsonin to act, phagocytosis might be obtained.

As it would be necessary to check vegetative growth in experiments that were to continue for 24 hours, the cells in the suspension of *Micrococcus aureus* (derived from a whitlow) were steamed for 10 minutes.* The suspension was then centrifuged to get rid of any small clumps that might have formed. The washed leucocytes were obtained in the manner recommended by Wright and Douglas, which has been described in these pages (*antea*, p.296). The proportions of corpuscles, bacterial suspension and serum were measured and treated as by these investigators excepting where otherwise noted.

The heated serum was mixed with the bacterial suspension in the proportion of 3 : 1 and sealed in a capillary tube which was heated for 20 hours at 37°. It was then mixed with the corpuscular suspension in the proportion of 4 : 3. A control test was made with the same serum, unheated, which had been kept

* When the temperature of a control tube was 94°.

at room temperature (17°). The tests were sealed in capillary tubes and heated at 37° for 30 minutes. Films were made and stained with the Leishman stain. The polynuclear leucocytes were enumerated and the staphylococci contained within were counted.

In the tabulated results that follow, the phagocytic index means the average number of bacteria englobed by a polynuclear white blood corpuscle, and the numbers within the brackets indicate the total number of the phagocytes that were counted. The brackets following the suspensions show the time during which the serum and suspension were in contact.

Opsonisation by heated serum.

Normal saline + suspension + corpuscles	No phagocytosis.*
Normal serum + suspension + corpuscles	Phagocytosis. Phagocytic Index, (22) = 25.
Heated serum + suspension (20 hours at 37°) + corpuscles.	Phagocytosis. Phagocytic Index, (22) = 25.

From this experiment it is clear that heating the serum for 15 minutes at 60° does not destroy the opsonins, but simply retards their activity. By allowing a sufficient time for the reaction to take place, the opsonisation is complete.

A second experiment was made two weeks later with the following results.

	Phagocytic Index.
Normal serum + suspension + corpuscles.....	(40) = 23
Normal serum + suspension (20 hours at 37°) + corpuscles.....	(25) = 24
Heated serum + suspension + corpuscles.....	(29) = 0
Heated serum + suspension (5 hours at 37°) + corpuscles.....	(25) = 10
Heated serum + suspension (20 hours at 37°) + corpuscles.....	(40) = 24

This also shows that the opsonin in time recovers from the effect of the heat to which it has been subjected, or to express it

* In the normal saline test, there were no bacteria within the leucocytes; one or two, here and there, were on the outside of the phagocytes, but as these had evidently adhered in drying the films they were ignored.

in a better way, the reaction between the opsonin and the products of the bacterium that has been retarded by heat is complete in at least 20 hours. Contact for 5 hours produced a partial recovery of the reaction. A similar experiment confirmed these results.

In order to vary the experiment, serum was diluted with normal saline in the proportion of 1 of the former to 4 of the latter, thus making a 20 % solution of serum. It was heated for 15 minutes at 50°, 60°, 65° and 70°, then mixed with the suspension of dead bacteria, sealed in capillary tubes and heated at 37° for 24 hours before being treated with the suspension of blood corpuscles. The proportion of dilute serum, bacterial suspension and corpuscles was the usual 3 : 1 : 3, and 30 minutes was the time given for phagocytosis to take place at 37°. In enumerating the bacteria ingested, it was evident that the results in some cases could only be approximate, for in certain of the tests the bacteria had agglutinated and many of the phagocytes had gathered round the clumps. Again, polynuclear leucocytes were seen over, under, or against clumps, making it impossible to tell how many cells were within the phagocyte. Furthermore, as in such cases, the cells were not uniformly distributed, every mobile leucocyte would not have the same chance of englobing a staphylococcus or even a small clump. When there is no clumping, the numbers of bacteria ingested vary within comparatively narrow limits, but when the microbes are agglutinated the variations are great and there are many phagocytes noted with no bacteria. Since one cannot count the bacteria within the phagocytes which have gathered around a large bacterial clump, even when, as is generally the case, they are gorged, it is only fair to ignore the polymorphonuclear corpuscles when they contain none, one or up to five cells and when they contain over fifty. The proof that such a discrimination is necessary is shown in the following table in the test with the diluted serum heated to 50°. The bacteria were clumped and the readings were—

Total count	(50) = 7
Omitting numbers under 6 and over 50 as above explained....	(11) = 31

The amended phagocytic index is identical with that of the unheated dilute serum, and that is what we should expect to find.

Opsonisation with diluted serum.

Dilute serum heated for 15 minutes at	Hours during which serum + suspension was heated at 37°.	Condition of free bacteria in the films.	Original Phagocytic Index of tests when amended.	Phagocytic Index.
Room temperature	0	distributed	.....	(25) = 31
50°	24	agglutinated	(50) = 7	(11) = 31
60°	24	agglutinated	(77) = 13	(35) = 33
65°	24	distributed	(50) = 4	(25) = 10
70°	24	distributed	.....	(25) = 0
60°	5	agglutinated	(25) = 19	(17) = 28
60°	0	distributed	.....	(25) = 0

Opsonisation with the same serum undiluted.

Heated at 60° + suspension + corpuscles	Phagocytic Index, (25) = 0
Not heated + suspension + corpuscles	Phagocytic Index, (20) = 25

The clumping that occurred in certain of these tests was pronounced, and strongly supports the idea that opsonisation is a phase of agglutination. We must distinguish between a deposition of a precipitate and the flocculation of the same. The precipitate formed by the action of the agglutinin (precipitin or opsonin) upon the agglutinable substance oozing through the bacterial membranes is deposited upon the capsules of the cells. This is probably opsonisation, and is the first phase of the complete phenomenon. The second is the flocculation of these deposited films, that is the agglutination of the opsonised bacteria under the further influence of the saline matter of the suspension. In the first test there was opsonisation without agglutination, because a sufficient time had not been given for the second phase to develop. In the second, third and sixth tests, even when the dilute serum had been heated, the time was sufficient for the phenomenon to be complete.

An alkaline citrate was used in diluting the serum for the purpose of hindering the clumping and thus regulating the

ingestion of the opsonised bacteria. This object, however, was not attained, for agglutination was manifest in the films. Probably the citrate was not present in sufficient amount to prevent the flocculating action of the common salt added with the corpuscular and bacterial suspensions. In the experiment, the serum was diluted with four parts of 1 % potassium citrate or of normal saline (0·6 % NaCl).

Opsonisation and Agglutination with dilute serum.

Serum diluted with	Treatment.	Hours during which serum + suspension was heated at 37°.	Condition of free bacteria in the films.	Original Phagocytic Index of tests when amended.	Phagocytic Index.
citrate.....	not heated	0	distributed	.....	(80)=14
,, ...	,,	24	agglutinated	(50)=25	(17)=28
,, ...	,,	48	agglutinated	(50)=39	(21)=33
,, ...	heated at 60°	0	distributed	.....	(25)=0
,, ...	,,	24	agglutinated	(50)=17	(20)=21
,, ...	,,	48	agglutinated	(50)=17	(8)=26
,, ...	heated at 65°	24	distributed	.....	(50)=4
,, ...	,,	48	distributed	.....	(75)=1
normal saline	heated at 60°	24	agglutinated	(50)=38	(22)=30
,, ,,	,,	48	agglutinated	(50)=35	(26)=35
,, ,,	,,	24*	agglutinated	(50)=34	(21)=35

Opsonisation with undiluted serum.

Serum (24 hours at 20°) + suspension + corpuscles.....	distributed	.....	(50)=20
Serum (24 hours at 37°) + suspension + corpuscles.....	distributed	.....	(50)=13
Serum, heated at 60° + suspension (15 hours at 37°) + corpuscles	agglutinated	(41)=26	(35)=22
Serum, heated at 60° + suspension (24 hours at 37°) + corpuscles.....	agglutinated	(50)=26	(30)=24

Without discussing the action of the citrate in hindering or in not assisting the opsonic, and probably also the agglutinative effect, it is evident that the experiments confirm what has already

* Serum heated before dilution.

been done, and emphasise the correspondence of opsonisation and agglutination. The citrated serum showed a certain opsonic effect within half-an-hour, but when the time of contact with the bacteria was increased, the agglutinative effect became manifest and simultaneously the opsonic effect was enhanced.

In the experiments with the diluted serum, it was curious that while normal serum had a phagocytic index of 25, the same serum, diluted to one-fifth, instead of an index of 5 had that of 31. The phagocytosis with the dilute serum was relatively six times greater than with the undiluted. This was also found by Wright and Douglas in experiments with unheated serum diluted with heated serum and with normal saline. In their experiments a three-fold dilution of serum increased the opsonic effect to a maximum. In the following, the same result was obtained.

THE EFFECT OF DILUTING NORMAL SERUM.

Serum not diluted	Phagocytic Index, (40)=25
Serum and normal saline 1 : 1	,, ,, (40)=28
,, ,, ,, 1 : 3	,, ,, (40)=29
,, ,, ,, 1 : 5	,, ,, (40)=28
,, ,, ,, 1 : 10	,, ,, (50)=23
,, ,, ,, 1 : 20	,, ,, (40)=14
,, ,, ,, 1 : 40	,, ,, (40)=8
,, ,, ,, 1 : 80	,, ,, (50)=6

It is known that in flocculation generally, potassium salts are more active than those of sodium. In the special case of agglutination, Friedberger* showed that the same law held, and Joos† admitted that suspensions of *Bact. typhi* were rather more slowly agglutinated by sodium chloride than by potassium or ammonium chlorides.

In three experiments with living staphylococci, opsonisation was more pronounced when potassium chloride was used in making the dilutions, the bacterial and the corpuscular suspensions.

* Cent. f. Bakt. i. xxx. 342(table).

† Cent. f. Bakt. i. xxx. 857.

Opsonisation in Solutions of Sodium and Potassium Chlorides.

		Phagocytic Index with Sodium chloride (0·6 %).	Potassium chloride (0·6 %).
6/11/05.	Serum diluted, 1-10.	(50)=10	(25)=18
9/11/05.	,, ,, 1-20.	(25)=11	(25)=18
14/11/05.	Serum undiluted.*	(25)=13	(50)=13
,,	Serum diluted, 1-3,	(25)=12	(50)=12
,,	,, ,, 1-7.	(25)=8	(25)=9
,,	,, ,, 1-12.	(25)=6	(50)=10
,,	,, ,, 1-18.	(25)=6	(25)=9
,,	,, ,, 1-25.	(25)=6	(25)=8
,,	,, ,, 1-32.	(25)=6	(50)=8
,,	,, ,, 1-40.	(25)=5	(25)=6

An agglutination test was made at the same time, but as clumping was pronounced with a dilution of 1-40, it was repeated upon the following day.

Agglutination of living Staphylococcus suspensions with normal serum diluted with Sodium and Potassium Chlorides (0·6 %).

Dilution of serum (1) to salt.	Sodium chloride; unheated serum.		Potassium chloride; unheated serum.		heated serum.	
	30 min.	60 min.	30 min.	60 min.	2 hours.	20 hours.
5	0	1	0	1	0	
10	1	2	1	2	0	
15	1	3	1	3	0	
20	1	3	1	3	0	
30	1	2	1	3	0	
40	1	2	1	3	0	complete
50	1	2	1	3	0	sedimentation
60	1	2	1	3	0	
80	1	2	1	3	0	
100	1	1	1	3	0	
140	0	1	1	3	0	
200	0	1	1	3	0	
Check	—	—	—	—	0	partial sedimentation

* The index of the undiluted serum is low. Variations in the opsonic power of the same individual are of common occurrence, although Urwick (Brit. Med. Journ. July 22, 1905, 173) writes, "The opsonic power of healthy people varies very slightly, or not at all, from day to day." Upon reading through the papers of Wright and Douglas, one finds similar variations, *e.g.*, Proc. Roy. Soc. 74 (499) 152, patient's serum 13/4/04, (20)=30·3; 15/4/04, (20)=10·05. Again, *op. cit.* 72 (483) 363, with A. E. W.'s serum, Expt. 3, tube 1, (9)=25·4, tube 2 (18)=16·0.

In the table, "0" means no agglutination visible, "1" represents small suspended floccules, "2" stands for a partial flocculent sedimentation, and "3" indicates a complete flocculent sedimentation with a faint opalescence. The small sedimentation tubes were kept at 37°, excepting in the case of the heated serum, which stood overnight at room temperature (20°).

We see that potassium chloride agglutinated suspensions of living staphylococci more readily than sodium chloride, and that heating the serum to 60° for 15 minutes prohibited the rapid action of the staphylococcus agglutinin. That the action of the heat was not to destroy the agglutinin was shown by the complete sedimentation of the tests at the end of 20 hours. The microscopical examination of the sediments after that time proved that the partial sedimentation of the check test was due to gravitation, for the bacteria were singly and in pairs. In all the tests which had been treated with heated serum small clumps were found in addition to the individual and paired cells. The influence of the dilution, in increasing the agglutination, as seen in the tests with 5-, 10- and 15-fold dilutions, is noteworthy in view of the fact that a relatively greater opsonic effect is obtained upon diluting serum (p.563).

The staphylococci lend themselves admirably to experiments such as these. They are comparatively large, they stain very deeply, and they are not easily bacteriolysed. Enumeration is easy, and the results can be relied upon. With other bacteria there is more or less swelling and disintegration within the leucocytes. Wright and Douglas noted this in their work upon the other bacteria.

Some of the bacteria that had been examined by these investigators were employed in an experiment which had for its object the recovery of the opsonic power after its retardation by heat. The serum was diluted with normal saline to make a 10 % solution. The films showed that a considerable bacteriolysis had occurred within the polynuclear white blood corpuscles. All stages between the normal bacterium and the smallest granule, the final visible result of bacteriolytic action, were seen. It was

clearly evident that any enumeration of the englobed bacteria would be misleading and of no value whatsoever. It was also found that *Bac. diphtheriæ* did not markedly differ from *Bac. typhi*, *Bac. coli*, *Bac. dysenteriæ* (Shiga), *Micr. melitensis*, or *Vib. choleræ*.* The opsonic power was reduced but never destroyed by heat.

Although the experiment was mainly intended to demonstrate the recovery of the opsonic power after heating, this could not be done for the following reason. When dead suspensions of *Bac. coli* or *Bac. typhi* are kept in contact for 24 hours with serum that has been heated at 60° the greater part of the staining power is lost. A considerable portion disappears by contact with heated (62·5°) serum and a small part with dilute serum that has been heated at 65° for 15 minutes. This factor contributed largely to prevent a reliable enumeration.† The observation raises the question, are the alexines of the serum destroyed by heat as easily as is generally supposed?

An experiment with *Bac. typhi* and *Bac. coli* showed that they were opsonised extensively by diluted serum heated at 60°, 62·5° and 65°. Phagocytosis in all cases was pronounced; the majority of the polynuclear leucocytes were gorged with bacteria in all stages of disintegration.

The instances of similarity between the opsonins and agglutinins, that I have brought forward, point strongly to the probable identity of the two. But there are other indications of similar behaviour. There is a rise, fall, and presumably higher base level of the agglutinins during the course of uncomplicated typhoid fever. This has been shown by Jorgensen‡ and by Iverson.§ It appears to be akin to the positive phase and

* These bacteria were obtained from Dr. Tidswell, of the Board of Health, Sydney.

† *Micr. melitensis* was the only micro-organism that showed an increased phagocytic index in the case of the long contact with heated serum, doubtless because it is more resistant to the action of the bacteriolytic bodies.

‡ Cent. f. Bakt. (Orig.) xxxviii. (1905), 475, 566.

§ Zeitsch. f. Hygiene, xlix. 1.

higher base level of opsonic action which, according to Wright, follow the inoculation of staphylococcus vaccine. Another similarity between opsonins and agglutinins is observed in the power which bacteria have of fixing and removing them both from solutions.

The points of possible difference between the opsonins and agglutinins relate to the experiments of others upon the simultaneous rise and fall of the two during immunisation. A very strong indictment against their identity is made by Wright and Douglas,* who say that "Normal human serum does not exert any characteristic agglutinating action upon the staphylococcus. Such agglutination as is obtained is not very sensibly increased under the influence of staphylococcus inoculations."†

The races of bacteria are known to vary in their agglutinability, and the staphylococcus is no exception. Otto‡ found that the agglutinability of races of the truly pathogenic staphylococci varied. Nicoles and Lesieur§ immunised a goat to a race of

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* Proc. Roy. Soc. lxxiv., 1904, 148.

† At another place they say that no parallel exists between the opsonic and agglutinating powers of the blood of tubercular patients. The agglutination of *Bac. tuberculosis* is difficult to determine. The bacterium grows very slowly, and in the cultures there are many old cells. It is a peculiarity of some bacteria that in their senescence they produce autoagglutinins and perhaps autolysins. Such has been demonstrated by Emmerich and Loew in the case of *Bac. pyocyaneus*. *Bac. tuberculosis* produces autoagglutinins, and it is difficult to distribute cultures uniformly in saline solutions. Wright and Douglas ground up their cultures in an agate mortar with a 0·1% solution of common salt and obtained a suspension of bacterial fragments in which the autoagglutinative action was in abeyance. Although this strength of salt is not the most favourable, it is still sufficient to enable agglutination to become manifest in an hour at 37°. But it is probable that any agglutination that did appear might have been a combination of true agglutination and autoagglutination, the latter being induced by the salts added with the serum. For this reason *Bac. tuberculosis* does not promise to be suitable for showing analogies between agglutination and opsonisation.

‡ Cent. f. Bact. (Orig.) xxxiv. 44.

§ *Ibid.* (Ref.) xxxi. 158.

Micr. aureus and found that the serum agglutinated the infected race in dilutions of 1-50, while with three other races one gave a characteristic reaction and two did not.

With regard to the action of normal human serum upon suspensions of the staphylococcus, very little has been done. Beitzke* tested 44 specimens of serum, chiefly of corpses. Seventeen of these did not agglutinate when used in dilutions of 1-10. Seven agglutinated in dilutions of 1-50, nine in 1-100, eight in 1-200, and one each in 1-500, 1-1000, 1-2000. He thus found that 61 % of the cases gave a characteristic reaction, and he considered that the agglutination of the staphylococcus by normal human blood was of remarkably frequent occurrence. It may be objected that the blood of cadavers can scarcely be called normal.

Wright† examined the blood of four normal men and found that they agglutinated suspensions of the staphylococcus when diluted up to 8- and 16-fold.

The experiment on p.564 shows that my own serum when diluted at least 200-fold agglutinated suspensions of the race of staphylococcus that has been used in this investigation. An agglutination in such dilution is undoubtedly characteristic.

Upon the quantity of agglutinable substance secreted or excreted by the bacterium depends the rapid or slow agglutinability with a normal serum. The thinnest film of precipitate, resulting from the reaction between agglutinin and agglutinable substance, will alter the nature of the bacterial surface. From being indifferent to the leucocytes, the bacteria will become positively chemotactic. The flocculating action of the salts, however, may not be sufficiently powerful to cause the thinnest films with the enveloped bacteria to run together into clumps. We can therefore expect to find that races of the staphylococcus while exhibiting a normal opsonisation may be very slowly agglutinated.

This was to a certain extent borne out by the examination of two races which I obtained from Dr. Tidswell, one of *Staphylo-*

* Cent. f. Bakt. (Ref.) xxxv. 709.

† Lancet, 29th March, 1902, p.874.

coccus pyogenes albus, the other *Staph. pyogenes aureus*. These races were compared with the race which had been used throughout these experiments under the name of *Micr. aureus* (Mig.). In testing the agglutinative power, the serum was used in dilutions varying from 1-10 to 1-100.

AGGLUTINATION AND OPSONISATION OF THREE RACES OF STAPHYLOCOCCUS.

	Agglutination visible microscopically in		Phagocytic Index.
	15 min.	4 hours.	
Micr. aureus, 24 hours at 37°..	1-100	—	(50)=26
Staph. aureus, 24 hours ,,	1-10	1-25	(75)=14
,, ,, 48 hours ,,	1-10	1-100	(30)=21
Staph. albus, 24 hours ,,	—	1-10	(75)=16
,, ,, 48 hours ,,	—	—	(30)=24

The experiment shows that a weak agglutination may be accompanied by a weak opsonisation, but when time is given for the bacteria to produce a greater quantity of agglutinable substance, the agglutination becomes stronger and at the same time the opsonic power is increased.

The investigation has shown that the opsonins and agglutinins are probably identical, inasmuch as they have many points of similarity, and probably no points of difference. The similarities are as follows :—

1. Staphylococcus opsonin and agglutinin are not destroyed at 60°; their powers are only temporarily in abeyance. Contact with the bacteria for 20 hours induces a recovery.

2. In dilute saline solutions the recovery of the opsonic power is accompanied by an agglutination of the bacteria.

3. Potassium chloride gives a greater agglutinative and a greater opsonic effect than sodium chloride.

4. Dilution of the serum with saline solutions increases the agglutinative and the relative opsonic effects.

5. Longer cultivation of a weak race of staphylococcus increases the agglutinability and the opsonisation of the cells.

Opsonisation appears to be the first phase of agglutination.